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CARDIAC INOTROPIC RESPONSES FROM CHANGES IN CARBON DIOXIDE TENSION IN THE CEPHALIC CIRCULATION OF ANAESTHETIZED DOGS

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SUMMARY

1. Experiments were performed on anaesthetized dogs to determine the effects of moderate changes in P_{CO_2} in the cephalic circulation on the inotropic state of the heart and on the reflex inotropic responses from changes in carotid sinus pressure.

2. The cephalic circulation was perfused, through the brachiocephalic and left subclavian arteries, with blood taken from the superior vena cava and equilibrated with various gas mixtures in a gas exchange unit. The carotid sinus regions were vascularly isolated and perfused with arterial blood at controlled pressures. Cardiac inotropic responses were assessed from the maximum rate of change of left ventricular pressure (dP/dt_{max}) with heart rate and mean aortic pressure held constant.

3. An increase in cephalic blood P_{CO_2} resulted in an increase in dP/dt_{max} and an increase in the unpaced heart rate. Small, graded changes in cephalic P_{CO_2} resulted in graded responses of dP/dt_{max}.

4. A change in carotid sinus pressure resulted in a significantly greater response of dP/dt_{max} when cephalic P_{CO_2} was high.

5. After interruption of the left cardiac sympathetic nerves, the responses of dP/dt_{max} to changes in cephalic P_{CO_2} and carotid sinus pressure were nearly abolished.

6. These results indicate that the tension of carbon dioxide in the cephalic circulation is likely to be of importance in the control of the inotropic state of the heart. They also imply that, in studies of cardiovascular reflex responses, it is important to control the carbon dioxide tension in the arterial blood.

INTRODUCTION

There is some evidence that changes in blood flow or blood gases in the cephalic circulation can result in cardiovascular responses. However, the changes in the cephalic circulation which have been induced in most previous studies have been well beyond those encountered in normal life. For example, Downing, Mitchell & Wallace (1963) and De Geest, Levy & Zieske (1965) clamped the arteries supplying the head of an animal and reported that there was an increased activity of the sympathetic nerves to the cardiovascular system. Hainsworth & Karim (1973) studied the effects of graded cephalic hypotension and reported that, unless the cephalic blood was

severely hypoxic, inotropic and vascular responses were obtained only at very low cephalic perfusion pressures. Previous studies of the cardiac responses to cephalic hypoxia and hypercapnia have also used very gross stimuli which were achieved by equilibration of blood with gas mixtures containing either no oxygen or 15–30% carbon dioxide (Downing *et al.* 1963; De Geest *et al.* 1965; Achtel & Downing, 1972). These experiments can have little relation to normal physiology. A further criticism of most of the previous work is that it is not possible to distinguish the direct effects upon the inotropic state of the heart from responses secondary to changes in heart rate and blood pressure (Furnival, Linden & Snow, 1970).

The present investigation was undertaken to determine whether moderate changes in P_{CO_2} in the cephalic circulation have an influence on the cardiac inotropic state, using a preparation in which heart rate and blood pressure could be controlled. Because the inotropic state of the heart is known to be influenced by baroreceptor reflexes (e.g. Hainsworth & Karim, 1972, 1973), we also undertook experiments to examine the interaction between the effects of changes in cephalic blood P_{CO_2} and changes in carotid sinus pressure.

METHODS

Dogs of weight 17–38 kg were anaesthetized with chloralose (100 mg kg^{-1}; Cambrian Chemicals Ltd., Croydon, Surrey) infused through a catheter inserted under local anaesthesia (2% amethocaine) through a saphenous vein so that the tip of the catheter lay in the inferior vena cava. Light surgical anaesthesia was maintained by further infusions of chloralose (about 10 mg kg^{-1} every 30 min). A mid-line incision was made in the neck, the trachea cannulated and the lungs ventilated by positive pressure with 40% oxygen in nitrogen by means of a Starling Ideal pump. The rate of the pump was 18 strokes min^{-1} and the stroke volume approximately 17 ml kg^{-1}. When the pleura was opened, an expiratory resistance was provided by immersing the expiratory outlet under 3 cm of water. This outlet was occasionaly occluded for 3 or 4 pump strokes to expand a collapsed lung.

The common carotid arteries and their branches were dissected free and, except for the lingual arteries which were left for subsequent cannulation, all branches were tied. Particular care was taken to avoid damage to the sinus nerves. In four of the dogs both vagosympathetic trunks were cut above the nodose ganglia.

The chest was opened by removing the fourth and fifth left ribs. A length of the thoracic aorta was mobilized by dividing the first four pairs of intercostal arteries between ligatures. The brachiocephalic and left subclavian arteries near their origins and a length of about 3 cm of the superior vena cava were dissected free of their attachments. The pericardium was opened and a pair of small silver ring electrodes were sewn on to the right atrial appendage to allow pacing of the heart. A miniature pressure transducer (Konigsberg, model P13) was passed through the left atrial appendage and the mitral valve and into the cavity of the left ventricle. The transducer was secured in position by a tie round the base of the appendage.

The perfusion circuit (capacity about 1·5 l) was filled with a mixture of approximately equal parts of heparinized blood, obtained from the animal 12–16 days prior to the experiment, and Krebs–Ringer solution. The haematocrit values of the stored blood and the dog's blood on the day of the experiment were $46 \pm 1{\cdot}8$% (mean ± S.E. of mean) and $42 \pm 1{\cdot}9$% respectively. The animal was given heparin (500 i.u. kg^{-1} then 50 i.u. kg^{-1} every 30 min, I.V.) and the perfusion circuit (Fig. 1) was connected to the animal. Temporary bypasses were connected between the proximal ends of a femoral and a carotid artery and a femoral and a jugular vein, to allow some blood flow to the cephalic region during cannulation procedures. A stainless-steel cannula was inserted into the cardiac end of the descending aorta and blood from this passed into a large reservoir bottle, to which a constant pressure of air was applied. Some blood from the reservoir perfused the distal end of the descending aorta, and some was pumped into a Perspex column, to which a constant pressure

of air was applied, and from which the blood was conveyed to a Y-shaped cannula inserted into the distal ends of the common carotid arteries. Blood from the region drained to a peripheral vein through cannulae inserted in the lingual arteries.

Cannulae were inserted into the cephalic ends of the left subclavian and the brachiocephalic arteries and the cephalic region of the dog was perfused, initially with arterial blood, at constant flow by means of a large adjustable roller pump. A wide-bore (8 mm) cannula was inserted into the cephalic end of the superior vena cava to allow blood from the cephalic region to drain by gravity into an open reservoir. Immediately that venous drainage was established, clamps were re-positioned

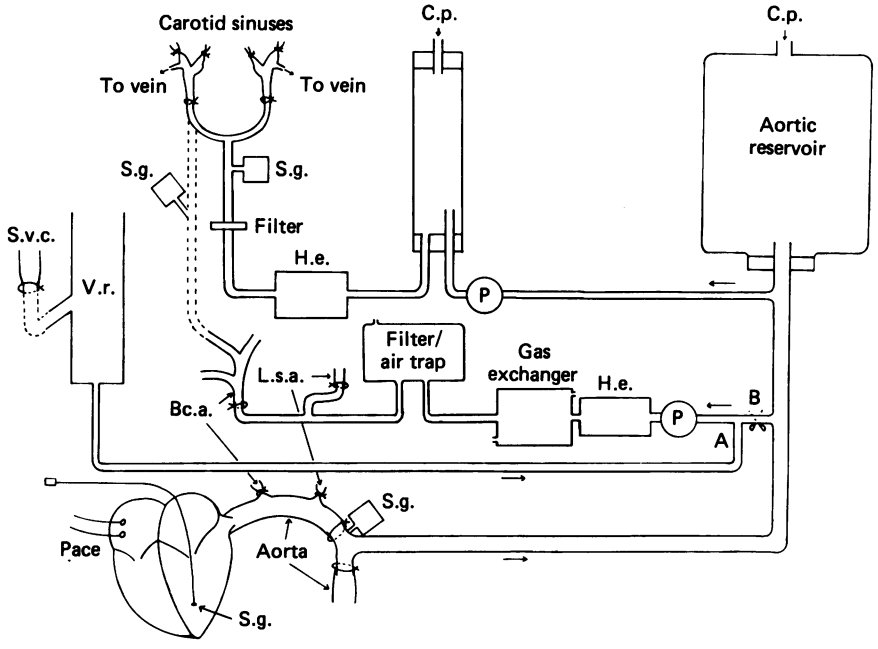

Fig. 1. Diagram of perfusion circuit. Cannula inserted in aorta conveys blood to circuit and to descending aorta. Both carotid sinuses perfused with arterial blood pumped through constant pressure chamber, heat exchanger and filter, into cannula inserted in both common carotid arteries. Cephalic circulation perfused with blood pumped through heat exchanger, gas exchanger, filter/air trap and into cephalic ends of brachiocephalic and left subclavian arteries. Initially cephalic circulation perfused with arterial blood (clamp at A). When superior vena cava cannulated, clamp transferred to B and cephalic circulation perfused with blood which drained into venous reservoir from superior vena cava. Abbreviations: c.p., constant pressure sources; s.g., strain gauges (Statham P23Gb transducers except in left ventricle; Konigsberg); h.e., heat exchangers; P, roller pumps; s.v.c., superior vena cava; v.r., venous reservoir; bc.a., brachiocephalic artery; l.s.a., left subclavian artery.

on the circuit so that the blood draining from the superior vena cava was pumped through a membrane gas exchanger (SciMed membrane lung) and into the brachiocephalic and left subclavian arteries.

Ventricular pressure, recorded using the Konigsberg transducer, was amplified and recorded on a direct-writing ultraviolet light oscillograph (S.E. Laboratories). The signal of ventricular pressure also passed to an analogue differentiator and an amplifier, and the rate of change of pressure (dP/dt) was recorded. The differentiator was calibrated using the method of Neal, Halpern & Reeves (1960). Other blood pressures were recorded using Statham transducers (P23Gb) connected to the cannulae in the descending aorta, femoral artery and carotid arteries, and to a cannula inserted in the cardiac end of a common carotid artery which was used to record cephalic perfusion pressure. Zero pressures

were recorded as the pressures obtained at the ends of each experiment with the ventricular transducer exposed and the other catheter tips free in air. Mean pressures were recorded, when required, by use of R–C networks with time constants of 2 s which were incorporated in the amplifiers.

The temperature of the animal, recorded using a thermistor probe (Yellow Springs Instruments) inserted to mid-oesophagus, was maintained at 36–38 °C by the use of electrical heaters under the operating table. The temperature of blood in the perfusion circuit was maintained at 37–38 °C by the use of heat exchangers in the circuit as shown in Fig. 1.

Arterial blood gases and pH in the systemic circulation and in the cephalic perfusate were estimated frequently using a Corning (Model 165) electrode system. Systemic arterial P_{O_2} was maintained above 10 kPa, if necessary by the addition of oxygen to the inspired gas, and P_{CO_2} and pH were adjusted to 4·6–6·0 kPa and 7·30–7·40 units by appropriate adjustment to the respiratory pump and intravenous infusions of molar sodium bicarbonate solution.

After connexion of the circuit, the animal was given suxamethonium chloride (0·5 mg kg^{-1} every 15 min) to prevent spontaneous respiratory movements. Chloralose infusions were continued at the same rate and after about 1 h the suxamethonium chloride was withheld long enough for the level of anaesthesia to be assessed.

Experimental procedure

The pressure in the aortic cannula was set to about 12 kPa by adjustment of the pressure of air above the aortic reservoir. It was held at a constant value for the duration of the experiment, if necessary by making adjustments to the aortic reservoir pressure. Except when responses of heart rate were studied, the heart was paced throughout the experiments at a constant rate of about 20 beats min^{-1} greater than that obtained at low carotid sinus pressure. The flow to the cephalic circulation was adjusted to set cephalic perfusion pressure to about 12 kPa. When necessary this pump was adjusted during the experiments to maintain cephalic perfusion pressure greater than 10 kPa. The height of the venous reservoir was adjusted so that the level of blood in it remained constant, indicating equality of inflow and outflow.

Systemic arterial blood gases were set to the values specified above and the tension of carbon dioxide in the cephalic perfusate was set to about 4 kPa by adjustment of the gas mixture supplying the gas exchanger. The preparation was allowed to stabilize for 10–15 min before records were taken. Cephalic P_{CO_2} was then increased in a single step by increasing the flow of carbon dioxide to the gas exchanger, and after about 5 min further records were taken before the carbon dioxide flow was decreased to its former value. Samples of systemic arterial and cephalic perfusate blood were taken at all levels of carbon dioxide flow and the blood gas tensions were adjusted as necessary before records were taken. The values of the variables obtained at the high cephalic P_{CO_2} were compared with the averages of the values at low P_{CO_2} before and after increasing it.

In some experiments the effects were recorded of changing cephalic P_{CO_2} in small steps. Experiments were also performed to determine the responses to changes in carotid sinus pressure at different levels of P_{CO_2} in the cephalic perfusate.

Values reported are of means ± S.E. of mean, and the significances of responses were assessed using the paired t test.

Critique of experimental approach

The method used in this study to separate the circulation to the cephalic part of the animal does not allow us to be precise as to the extent of the region which was subjected to the changes in P_{CO_2}. The cephalic region was perfused through the brachiocephalic and left subclavian arteries and drained from the superior vena cava. The evidence that there was adequate separation of cephalic circulation from caudal circulation is, first, that inflow and outflow to the region were approximately equal and, secondly, that large changes in cephalic blood P_{CO_2} had a relatively small effect on systemic blood gases.

The blood supply to the brain of the dog is normally provided by both vertebral and carotid arteries (Wellens, Wonters, Reese, Beirnaert & Reneman, 1975; Gillilan, 1976). In our preparations, the major supply would have been through the vertebral arteries since both common carotid arteries and their branches were tied to isolate the carotid region to control pressure and gas tensions to the baroreceptors and chemoreceptors. In the dog, an adequate supply of blood to the whole brain can be obtained from the vertebral arteries. We confirmed this during the connexion of the perfusion

circuits. When the common carotid arteries were tied, but before tying the internal and external carotid arteries, the pressure in the carotid artery distal to the tie was only a little lower than that proximal to the tie. During the course of the experiments, the rate of the cephalic perfusion pump was adjusted, if necessary, to maintain the perfusion pressure about 12 kPa. Therefore, due to a high perfusion pressure and a high oxygen tension in the cephalic perfusate (20·1 ± 2·1 kPa), the responses obtained could not have been due to ischaemia or hypoxia and were almost certainly due to the change in P_{CO_2} or pH.

It was not possible in these experiments to determine whether the change in P_{CO_2} or the resulting change in pH was the effective stimulus. To induce independent changes in P_{CO_2} and pH would have necessitated a more complete isolation of the cephalic circulation to ensure that, when the necessary infusions of acidic or alkaline solutions were made, the non-respiratory changes of pH would have been confined to the cephalic region. Furthermore, the question of whether P_{CO_2} or pH is the effective stimulus is complicated by the fact that changes in P_{CO_2} in the arterial blood result in changes in pH in the cerebrospinal fluid (Loeschcke, 1982) and therefore, even if the blood levels of pH and P_{CO_2} have been controlled, this would not necessarily have proved which was actually the effective stimulus.

The region which was included in the cephalic perfusion would have included not only intracranial structures, but also extracranial head, neck and forelimbs. We cannot, therefore, be certain of the structures which were responsible for inducing the responses to the changes in P_{CO_2}. The carotid baroreceptors and chemoreceptors were excluded, but there are a small number of chemoreceptors, situated at the origins of the subclavian arteries (Coleridge, Coleridge & Howe, 1970), which would have been subjected to the changes in P_{CO_2}. However, these are unlikely to have made a significant contribution to the responses partly because at high oxygen tensions, as were used in the present study, aortic chemoreceptors are not very sensitive to carbon dioxide (Sampson & Hainsworth, 1972). Also, in some experiments the vagosympathetic trunks were cut, which would have denervated these chemoreceptors, and responses were not significantly different (see Results). Other known sensory regions which might have participated in the responses are the nasopharynx (Angell James & Daly, 1972) and larynx (Boushey, Richardson & Widdicombe, 1972). However, there is no evidence that nasopharyngeal receptors respond to changes in P_{CO_2} and stimulation of laryngeal receptors predominantly causes respiratory responses (Boushey & Richardson, 1973) which, in the present experiments, were prevented from occurring. Furthermore, in some of the present experiments, in which the vagus nerves were cut above the level of the nodose ganglia to denervate laryngeal receptors, responses were not different.

It seems most likely, therefore, that the responses to changes in cephalic P_{CO_2} were due predominantly to an effect on the brain or upper spinal cord. We were not able to localize the sensory region any further. One obvious possibility is that the responses resulted from stimulation of the so-called medullary chemoreceptors (Loeschcke, 1982) which are sensitive to the changes in pH in the cerebrospinal fluid resulting from changes in cerebral blood P_{CO_2}. However, the evidence that physiological changes in pH or P_{CO_2} in the cerebrospinal fluid can result in cardiovascular responses is not unequivocal. Certainly, increases in blood pressure have been obtained when the ventral surface of the medulla was subjected to increases in P_{CO_2} or hydrogen ion concentration (Schlaefke, See & Loeschcke, 1970; Szulczyk & Trzebski, 1976; Lioy, Hanna & Polosa, 1981). However, the stimuli applied have usually been gross, with values of P_{CO_2} of over 11 kPa and pH of 6·8 or less having been used. We would suggest, therefore, that while the medullary chemoreceptors may have induced the responses to changes in cephalic P_{CO_2} in the present experiments, this is not yet firmly established and the mechanism is still unknown.

RESULTS

Inotropic response to large changes in cephalic P_{CO_2} at low carotid sinus pressure

Experiments were carried out in seventeen dogs. The tension of carbon dioxide in the cephalic perfusate was changed from 4·1 ± 0·2 kPa to 9·7 ± 0·6 kPa and back to 4·7 ± 0·2 kPa. The corresponding values of pH were 7·45 ± 0·09, 7·15 ± 0·09 and 7·42 ± 0·09 units. During the tests P_{O_2} in the perfusate did not change significantly

at 20·1 ± 2·1 kPa. Heart rate, mean aortic pressure and carotid sinus pressure were held constant at 207 ± 6·6 beats min^{-1}, 11·7 ± 0·2 kPa and 8·3 ± 0·2 kPa respectively.

An increase in cephalic P_{CO_2} consistently resulted in an increase of dP/dt_{max} (e.g. Fig. 2). The mean response was an increase from 488 ± 31 kPa s^{-1} to 712 ± 60 kPa s^{-1} (+49 ± 14 %; $P < 0·005$). The corresponding values of left ventricular peak and

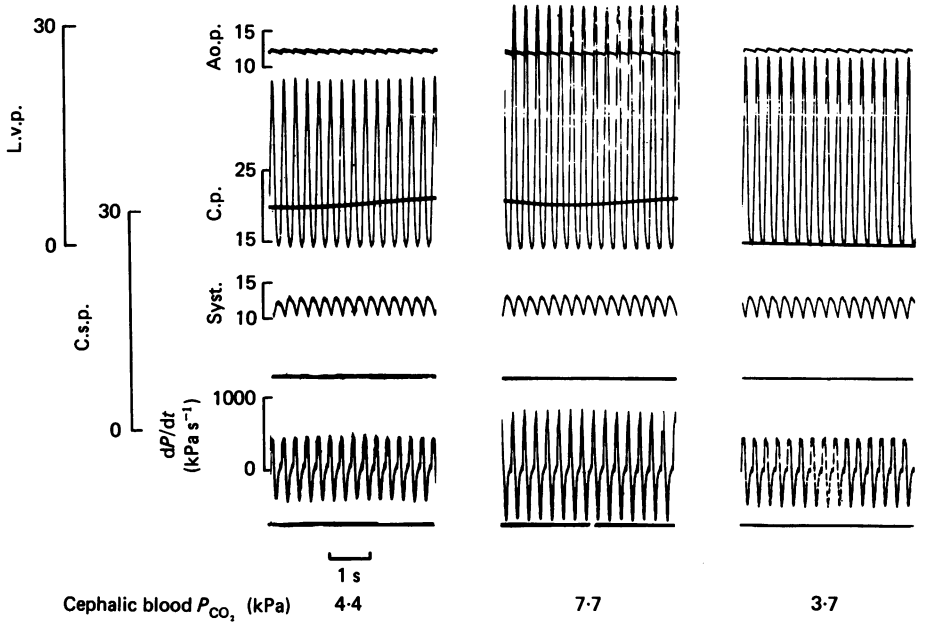

Fig. 2. Responses to single large step changes in cephalic blood P_{CO_2}. Each panel obtained in steady state obtained 5 min after any change in the P_{CO_2} in the cephalic perfusate. Records from top to bottom are of aortic pressure (ao.p.), left ventricular pressure (l.v.p.), cephalic perfusion pressure (c.p.), systemic arterial pressure recorded in femoral artery (syst.), carotid sinus pressure (c.s.p.), rate of change of left ventricular pressure (dP/dt), and datum line. All pressures measured in kPa and dP/dt in kPa s^{-1}.

end-diastolic pressures were 19·9 ± 1·2 kPa and 0·91 ± 0·09 kPa at low cephalic P_{CO_2} and 26·0 ± 2·8 kPa and 0·91 ± 0·10 kPa at the high P_{CO_2}. The change in peak pressure (mean +27 ± 8 %) was significant ($P < 0·005$) but the change in end-diastolic pressure was not.

Effect of cutting cervical vagosympathetic trunks. In four of the dogs, both cervical vagosympathetic trunks were cut above the nodose ganglia. The values of dP/dt_{max}, at low and high values of cephalic P_{CO_2}, were not significantly different from values obtained in dogs with intact vagi. All results, therefore, have been analysed together.

Inotropic responses to graded changes in cephalic P_{CO_2}

In four dogs, five tests were performed of increasing and decreasing cephalic P_{CO_2} in four or five steps of about 1 kPa from hypocapnic to hypercapnic and back to hypocapnic levels. Fig. 3 shows some of the traces from one dog of the responses to

increases in P_{CO_2}. In all experiments, responses of dP/dt_{max} were obtained with each change of cephalic P_{CO_2}, both below and above the normocapnic values. dP/dt_{max} showed an approximately linear increase from 394 ± 61 kPa s^{-1} at a cephalic P_{CO_2} of 4·0 kPa to 480 ± 54 kPa s^{-1} at 7·0 kPa. Thus, on average, dP/dt_{max} increased by 29 kPa s^{-1} for each 1 kPa increase in cephalic P_{CO_2}.

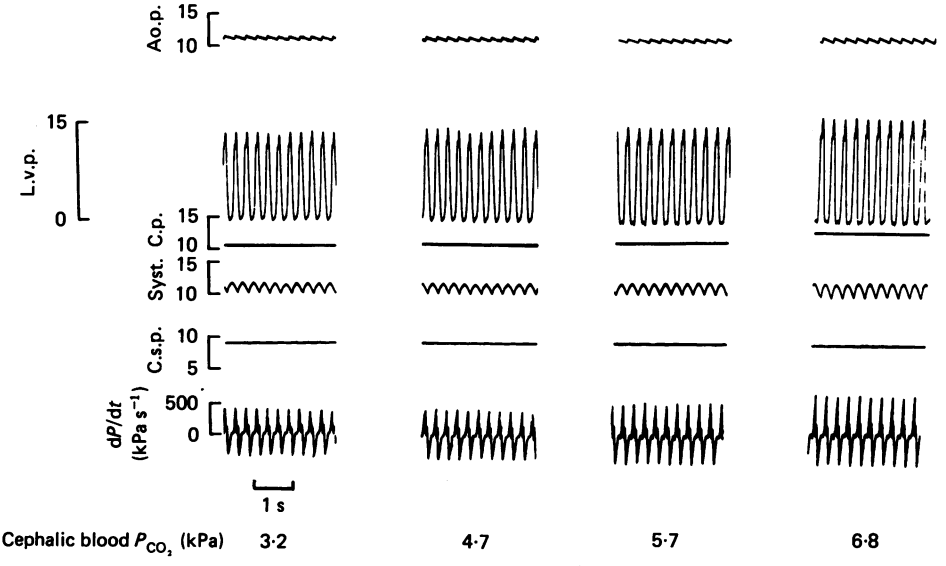

Fig. 3. Responses to graded changes in cephalic blood P_{CO_2}. Traces and abbreviations as in Fig. 2.

Inotropic responses to large changes in carotid sinus pressure at low and high cephalic P_{CO_2}

In the seventeen dogs in which the effects of large changes in cephalic P_{CO_2} were studied, carotid sinus pressure was increased in a large single step, over the entire baroreceptor sensitivity range, at both low and high levels of cephalic P_{CO_2}. An example of the responses is shown in Fig. 4. The highest value of dP/dt_{max} was obtained at low carotid sinus pressure and high cephalic P_{CO_2}. At the high carotid pressure there was little difference in the values of dP/dt_{max} obtained at high and low cephalic P_{CO_2}. At the low cephalic P_{CO_2} ($4·1 \pm 0·2$ kPa), the average response to an increase in carotid sinus pressure from $8·3 \pm 0·2$ kPa to $27·7 \pm 0·7$ kPa was a decrease in dP/dt_{max} from 488 ± 31 kPa s^{-1} to 403 ± 29 kPa s^{-1}. At the high cephalic P_{CO_2} ($9·7 \pm 0·55$ kPa), the same change in carotid sinus pressure decreased dP/dt_{max} from 712 ± 60 kPa s^{-1} to 456 ± 31 kPa s^{-1}. These results also indicate that increases in cephalic P_{CO_2} at the low carotid sinus pressure resulted in responses of dP/dt_{max} (average $+49 \pm 14$ %), which were significantly ($P < 0·005$) greater than the responses at high carotid pressure (average $+13·3 \pm 4·1$ %).

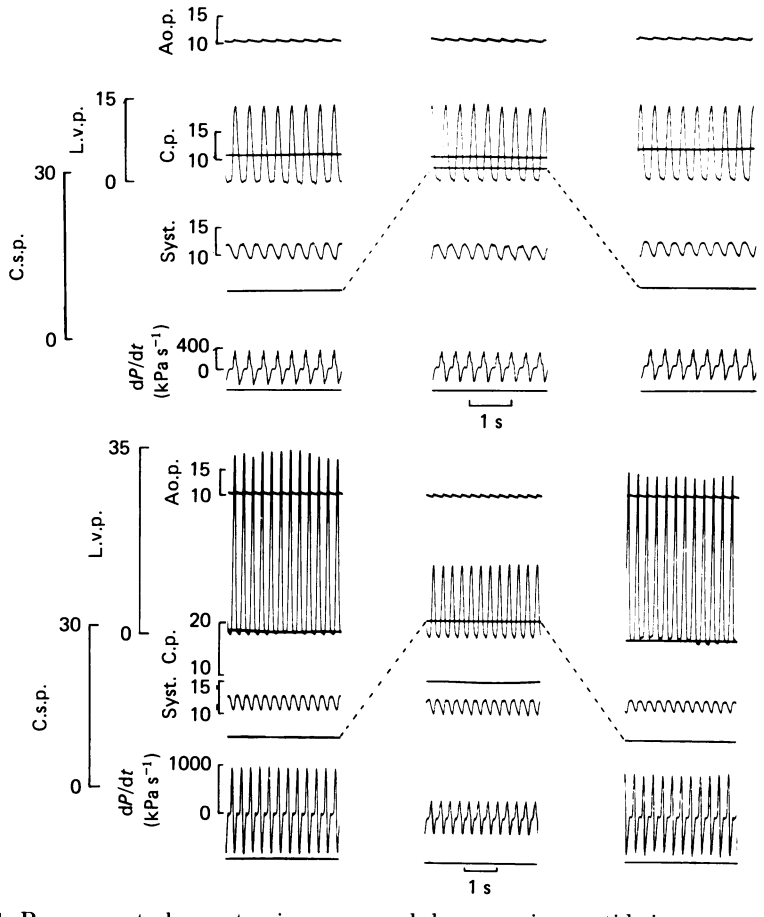

Fig. 4. Responses to large step increases and decreases in carotid sinus pressure. Upper traces obtained during perfusion of cephalic circulation with blood with P_{CO_2} of 4·9 kPa and lower traces obtained in same dog at P_{CO_2} of 8·4 kPa. Traces and abbreviations as in Fig. 2.

Inotropic responses to graded changes in carotid sinus pressure at low and high cephalic P_{CO_2}

In five dogs, carotid sinus pressure was increased and decreased in steps of about 4·5 kPa from about 8 kPa to about 30 kPa at low, high and again at low levels of cephalic P_{CO_2}. The results from one dog have been plotted (Fig. 5) and show that the responses to changes in carotid sinus pressure were greater at the higher cephalic P_{CO_2}. The difference between the plots was greatest over the lower carotid pressures. The relationships between dP/dt_{max} and carotid sinus pressure, obtained at different levels of cephalic P_{CO_2}, were analysed by use of polynomial (cubic) regression analyses (Snedecor & Cochran, 1967). A computer program (Texas Instruments) was used to fit the data points, by least-squares criterion, to equations of the form

$$y = A_1 + A_2x + A_3x^2 + A_4x^3,$$

where A_1–A_4 are constants and y and x are values of dP/dt_{max} and carotid sinus pressure respectively. The maximum slopes of these curves were calculated from the first differential, at the point at which the second differential was equal to zero (i.e. the inflexions of the curves). The maximum and minimum values of dP/dt_{max} and the maximum slopes of the curves, obtained at the two levels of cephalic P_{CO_2}, have

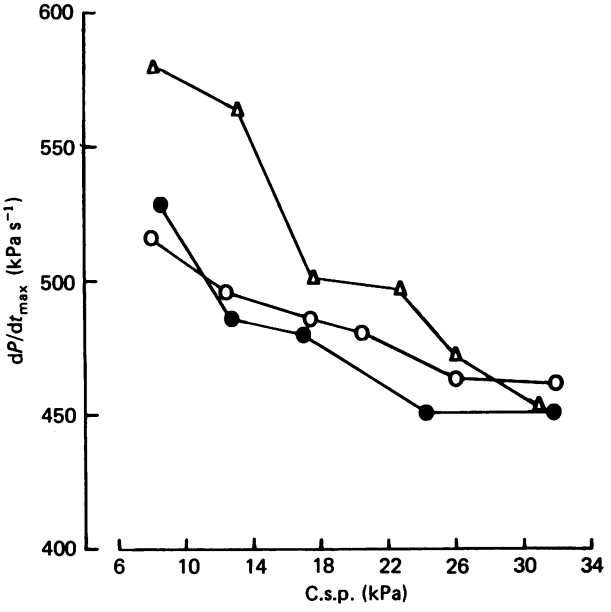

Fig. 5. Responses to graded changes in carotid sinus pressure (c.s.p.) at different levels of cephalic blood P_{CO_2}. ●, values obtained initially at P_{CO_2} of 4·2 kPa; △, values obtained after cephalic P_{CO_2} had been at 7·2 kPa for 5 min; ○, values obtained 5 min after reducing cephalic P_{CO_2} to 3·1 kPa.

been listed in Table 1. This shows that not only is the effect of cephalic P_{CO_2} predominant at low carotid sinus pressures but also that the response to changing carotid sinus pressure, expressed as the maximum slope of the stimulus–response curve, or maximum 'gain' of the reflex, is significantly greater at the high cephalic P_{CO_2}.

Effect of crushing left ansa subclavia on inotropic responses to changes in cephalic P_{CO_2} and carotid sinus pressure

The results, obtained from five dogs, are summarized in Table 2. After crushing the left cardiac sympathetic nerves, the responses of dP/dt_{max} to changes in both cephalic P_{CO_2} and carotid sinus pressure were nearly abolished. The values of dP/dt_{max} after denervation were close to those obtained before denervation at high carotid pressure and low cephalic P_{CO_2}.

Chronotropic responses to large single step changes in cephalic P_{CO_2}

The response of the unpaced heart rate to a change in cephalic P_{CO_2} was studied in eighteen dogs. Three of these dogs were also used to study inotropic responses and,

TABLE 1. Responses of dP/dt_{max} to graded changes in carotid sinus pressure at different levels of cephalic blood P_{CO_2}

	Low cephalic P_{CO_2}			High cephalic P_{CO_2}		
	dP/dt_{max}			dP/dt_{max}		
Dog	Low c.s.p.	High c.s.p.	Maximum slope	Low c.s.p.	High c.s.p.	Maximum slope
11	815	675	16·2	925	675	25·1
12	515	465	5·0	580	470	22·5
22	560	510	2·6	770	540	50·0
23	314	216	11·0	408	265	16·3
24	485	430	7·1	535	435	25·0
Mean	538	459	14·6	644	477	37·0
S.E. of mean	81	74	6·5	91	67	10·4
P				$< 0·02$	$> 0·5$	$< 0·02$

Low cephalic P_{CO_2} indicates values obtained during perfusion of the cephalic circulation with blood in which the P_{CO_2} was $3·8 \pm 0·4$ kPa and high cephalic P_{CO_2}, $7·3 \pm 0·5$ kPa. Values of dP/dt_{max}, given in kPa s^{-1}, were determined at low ($7·7 \pm 0·2$ kPa) and high ($28·8 \pm 0·7$ kPa) values of carotid sinus pressure (c.s.p.). Maximum slope given in kPa s^{-1} kPa^{-1} and calculated from polynomial regression analysis (see text). Levels of significance relate to paired comparisons of values obtained at high and low cephalic P_{CO_2}.

TABLE 2. Responses of dP/dt_{max} to changes in carotid sinus pressure at different levels of cephalic blood P_{CO_2} before and after crushing left ansa subclavia

	Before crushing				After crushing			
	Low P_{CO_2}		High P_{CO_2}		Low P_{CO_2}		High P_{CO_2}	
Dog	Low c.s.p.	High c.s.p.	Low c.s.p.	High c.s.p.	Low c.s.p.	High c.s.p.	Low c.s.p.	High c.s.p.
13	487	467	758	480	454	453	423	411
14	240	189	335	223			217	187
15	600	540	700	600	538	540	530	515
16	473	390	908	450	442	443	461	390
17	395	369	549	381	435	420	438	423

All values listed are of dP/dt_{max} (kPa s^{-1}) obtained before and after crushing the left ansa subclavia. Values obtained at low (3–4 kPa) and high (7–8 kPa) values of cephalic blood P_{CO_2} and at low (8 kPa) and high (30 kPa) values of carotid sinus pressure (c.s.p.).

in the remaining fifteen, chronotopic responses only were studied. In fourteen of the dogs an increase in cephalic P_{CO_2} resulted in an increase in heart rate, and in four dogs heart rate decreased. The average values of heart rate at low and high cephalic P_{CO_2} were $170 \pm 7·6$ beats min^{-1} and $182·5 \pm 7·9$ beats min^{-1}. The difference was significant ($P < 0·01$). In twelve dogs the vagosympathetic trunks were cut, but the values of heart rate at low and high values of cephalic P_{CO_2} were not significantly different from the values in the six dogs with intact vagi.

DISCUSSION

The results of the present experiments have shown, for the first time, that moderate changes in P_{CO_2} and pH in the cephalic perfusate influence the inotropic state of the heart. Previous work in which cephalic P_{CO_2} was changed (Downing *et al.* 1963; De Geest *et al.* 1965) used such large changes in P_{CO_2} that the results can have very little physiological significance. Furthermore, in the previous studies the methods used to assess the inotropic state of the heart were not adequate to distinguish between a direct effect on the heart and an indirect effect due to changes in blood pressure or heart rate (Furnival *et al.* 1970). In the present study we defined the efferent pathway of the response by showing that it was almost totally abolished by cutting the left ansa subclavia, which contains most, although not all, of the efferent sympathetic nerves to the left ventricular myocardium (Randall & Rohse, 1956). The effect on the cardiac inotropic state, brought about by an increase in the level of carbon dioxide in the cephalic circulation and mediated by the efferent sympathetic nerves, is thus opposite to the direct effect of CO_2 on the heart, which results in a negative inotropic effect (Bos, Drake & Noble, 1979).

An increase in cephalic P_{CO_2} also resulted in a small, but significant increase in the heart rate. This response was not significantly different in those dogs in which the vagus nerves had been cut. It was not related to the initial heart rate; increases or decreases in heart rate could be obtained in dogs with either high or low initial heart rates. These experiments indicate that the tachycardia is due mainly to an increase in efferent sympathetic nerve activity.

The magnitude of the response of dP/dt_{max} to a change in cephalic P_{CO_2} at the low carotid sinus pressure was similar to the response to a change in carotid sinus pressure at high cephalic P_{CO_2}. However, although the two mechanisms seemed approximately equipotent, the carotid sinus reflex was dominant. At the high carotid pressure, the response to a change in cephalic P_{CO_2} was nearly abolished, whereas a relatively large response to a change in carotid pressure was obtained at all levels of cephalic P_{CO_2}. The experiments of crushing the sympathetic nerves to the heart provided evidence that, when carotid sinus pressure was high, the activity in the efferent cardiac sympathetic nerves was almost totally inhibited at all levels of cephalic P_{CO_2}.

The two mechanisms (cephalic P_{CO_2} and baroreceptors) interact such that the response of dP/dt_{max} to the combined increase in cephalic P_{CO_2} and a decrease in carotid sinus pressure ($+309$ kPa s^{-1}) is more than twice the sum of the individual responses to an increase in cephalic P_{CO_2} at high carotid pressure ($+53$ kPa s^{-1}) and a decrease in carotid pressure at low cephalic P_{CO_2} ($+85$ kPa s^{-1}). This interaction results in an increase in the 'gain' of the carotid sinus reflex when cephalic P_{CO_2} is increased; the maximum slope of the relationship between dP/dt_{max} and carotid sinus pressure was significantly greater when cephalic P_{CO_2} was high. The site of the interaction could be in the central nervous system and possibly in the medulla. Some observations in support of this come from experiments of Wennergren & Oberg (1980), who found that various chemicals applied to the ventral surface of the medulla resulted in changes in blood pressure and in the response of blood pressure to carotid occlusion. Also, McAllen, Neil & Loewy (1982) applied an excitotoxic drug to the glycine-sensitive area described by Feldberg & Guertzenstein (1976) and found that,

during the stimulation phase, the baroreceptor reflex was enhanced. However, although the interaction reported by Wennergren & Oberg (1980) and McAllen *et al.* (1982) may have been the same as that reported in the present work, there is no evidence that it is, and neither the site of action of the cephalic carbon dioxide stimulus nor the site of interaction with the baroreceptor reflex is known.

The significance of the findings of the present experiments is also open to conjecture. Although the response to a large change in cephalic P_{CO_2} is of a similar magnitude to the response to a change in carotid sinus pressure, in normal life the stimulus to the carbon dioxide sensitive areas would change much less than the stimulus to baroroceptors; under most circumstances P_{a,CO_2} remains nearly constant. Also, in the intact body carbon dioxide would be likely to influence the heart by other mechanisms. For example, carbon dioxide acts directly on the heart to decrease the inotropic state (Bos *et al.* 1979) and, if carotid chemoreceptors are stimulated, there would be a reflex negative inotropic response (Hainsworth, Karim & Sofola, 1979). Nevertheless changes in P_{a,CO_2} do occur when ventilation is altered and in various disease states. Also because of the interaction with, at least, the baroreceptor reflex, in physiological studies of cardiovascular reflex responses it is clearly important to maintain normal levels of arterial P_{CO_2} and to prevent it from changing during an experiment.

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